

residues 117 to 185 of SEQ ID NO: 1
residues 186 to 248 of SEQ ID NO: 1
residues 29 to 73 of SEQ ID NO: 3
residues 74 to 116 of SEQ ID NO: 3
residues 117 to 185 of SEQ ID NO: 3
residues 186 to 248 of SEQ ID NO: 3
residues 1 to 32 of SEQ ID NO: 5
residues 33 to 75 of SEQ ID NO: 5
residues 76 to 144 of SEQ ID NO: 5
residues 145 to 210 of SEQ ID NO: 5
residues 34 to 80 of SEQ ID NO: 7
residues 81 to 140 of SEQ ID NO: 7
residues 33 to 79 of SEQ ID NO: 8
residues 80 to 119 of SEQ ID NO: 8
residues 120 to 161 of SEQ ID NO: 8
residues 32 to 91 of SEQ ID NO: 21
residues 25 to 84 of SEQ ID NO: 22
residues 29 to 94 of SEQ ID NO: 23
residues 31 to 85 of SEQ ID NO: 24
residues 1 to 23 of SEQ ID NO: 25
residues 1 to 17 of SEQ ID NO: 26
residues 1 to 28 of SEQ ID NO: 27

(ii) a homologue of (i);

(iii) a polypeptide containing a relative cysteine spacing of C-2X-C-3X-C-(10-12)X-C-3X-C-3X-C (SEQ ID NOs:37-39) wherein X is any amino acid residue, and C is cysteine;

(iv) a polypeptide containing a relative cysteine and tyrosine/phenylalanine spacing of Z-2X-C-3X-C-(10-12)X-C-3X-C-3X-Z wherein X is any amino acid residue, and C is cysteine, and Z is tyrosine or phenylalanine;

(v) a polypeptide containing a relative cysteine spacing of C-3X-C-(10-12)X-C-3X-C wherein X is any amino acid residue, and C is cysteine;

(vi) a polypeptide with substantially the same spacing of positively charged residues relative to the spacing of cysteine residues as (i); and

(vii) a fragment of the polypeptide of any one of (i) to (vi) which has substantially the same antimicrobial activity as (i).

2. (Amended) An isolated or purified protein containing at least one polypeptide fragment according to claim 1, wherein said polypeptide fragment has a sequence selected from ~~within a sequence comprising SEQ ID NO: 1, SEQ ID NO: 3, or SEQ ID NO: 5.~~

3. (Amended) An isolated or purified protein having a sequence selected from SEQ ID NO:1, SEQ ID NO: 3, or SEQ ID NO:5.

7. (Amended) A transgenic plant ~~[harbouring]~~harboring a DNA construct according to claim 6.

9. (Amended) The transgenic plant according to claim 7, wherein said plant is selected from the group consisting of: maize, banana, peanut, field peas, sunflower, tomato, canola, tobacco, wheat, barley, oats, potato, soybeans, cotton, carnations, roses, ~~[or]~~and sorghum.

10. (Amended) ~~The [R]reproductive material of [a]the transgenic plant according to claim 7.~~

11. (Amended) ~~A composition comprising an antimicrobial protein according to claim 1 together with an agriculturally acceptable carrier diluent or excipient.~~

12. (Amended) A composition comprising an antimicrobial protein according to claim 1 together with a pharmaceutically-acceptable carrier diluent or excipient.

13. (Amended) ~~A method of controlling microbial infestation of a plant, the method comprising:~~

(i) ~~treating said plant with an antimicrobial protein according to claim 1 [or a composition according to claim 11; or~~

~~(ii) introducing a DNA construct according to claim 6 into said plant].~~

14. (Amended) A method of controlling microbial infestation of a mammal[ian animal], said[the] method comprising treating the [animal]mammal with an antimicrobial protein according to claim 1 [or a composition according to claim 12].

15. (Amended) The method of claim 14, wherein said mammal[ian animal] is a human.

16. (Amended) A method of preparing an antimicrobial protein, [which]said method [comprises the steps of:] comprising:

- a) obtaining or designing an amino acid sequence which forms a helix-turn-helix structure;
- b) replacing individual residues to achieve substantially the same distribution of positively charged residues and cysteine residues [as in one or more of the amino acid sequences shown in figure 4]; and
- c) [synthesising]synthesizing a protein comprising said amino acid sequence chemically or by recombinant DNA techniques in liquid culture[; and
- d) if necessary, forming disulphide linkages between said cysteine residues].

Please add the following claims:

17. The protein fragment of Claim 1, wherein said protein fragment is a polypeptide containing a relative cysteine and tyrosine/phenylalanine spacing of Z-2X-C-3X-C-(10-12)X-C-3X-C-3X-Z (SEQ ID NOs:34-36) wherein X is any amino acid residue, and C is cysteine, and Z is tyrosine or phenylalanine.

18. The protein fragment of Claim 1, wherein said protein fragment is a polypeptide containing a relative cysteine spacing of C-2X-C-3X-C-(10-12)X-C-3X-C-2X-C (SEQ ID NOs:31-33) wherein X is any amino acid residue, and C is cysteine.

19. The protein fragment of Claim 1, wherein said protein fragment is selected from the group consisting of:

- residues 29 to 73 of SEQ ID NO: 1;
- residues 74 to 116 of SEQ ID NO: 1;
- residues 117 to 185 of SEQ ID NO: 1;
- residues 186 to 248 of SEQ ID NO: 1;
- residues 29 to 73 of SEQ ID NO: 3;
- residues 74 to 116 of SEQ ID NO: 3;
- residues 117 to 185 of SEQ ID NO: 3;
- residues 186 to 248 of SEQ ID NO: 3;
- residues 33 to 75 of SEQ ID NO: 5;
- residues 76 to 144 of SEQ ID NO: 5;

residues 145 to 210 of SEQ ID NO: 5;
residues 34 to 80 of SEQ ID NO: 7;
residues 81 to 140 of SEQ ID NO: 7;
residues 33 to 79 of SEQ ID NO: 8;
residues 80 to 119 of SEQ ID NO: 8;
residues 120 to 161 of SEQ ID NO: 8;
residues 32 to 91 of SEQ ID NO: 21;
residues 25 to 84 of SEQ ID NO: 22;
residues 29 to 94 of SEQ ID NO: 23;
residues 31 to 85 of SEQ ID NO: 24; and
residues 1 to 23 of SEQ ID NO: 25.

20. The protein fragment of Claim 1 which is truncated, but wherein said truncated protein fragment has substantially the same antimicrobial activity as the nontruncated protein fragment.

21. A protein fragment according to Claim 1, wherein the protein fragment is a homologue of a protein fragment selected from the group consisting of:

residues 29 to 73 of SEQ ID NO: 1;
residues 74 to 116 of SEQ ID NO: 1;
residues 117 to 185 of SEQ ID NO: 1;
residues 186 to 248 of SEQ ID NO: 1;
residues 29 to 73 of SEQ ID NO: 3;
residues 74 to 116 of SEQ ID NO: 3;
residues 117 to 185 of SEQ ID NO: 3;
residues 186 to 248 of SEQ ID NO: 3;
residues 33 to 75 of SEQ ID NO: 5;
residues 76 to 144 of SEQ ID NO: 5;
residues 145 to 210 of SEQ ID NO: 5;
residues 34 to 80 of SEQ ID NO: 7;
residues 81 to 140 of SEQ ID NO: 7;
residues 33 to 79 of SEQ ID NO: 8;
residues 80 to 119 of SEQ ID NO: 8;

residues 120 to 161 of SEQ ID NO: 8;
residues 32 to 91 of SEQ ID NO: 21;
residues 25 to 84 of SEQ ID NO: 22;
residues 29 to 94 of SEQ ID NO: 23;
residues 31 to 85 of SEQ ID NO: 24; and
residues 1 to 23 of SEQ ID NO: 25.

22. A protein fragment having antimicrobial activity, wherein said protein fragment is selected from the group consisting of:

residues 1 to 32 of SEQ ID NO: 5;
residues 1 to 23 of SEQ ID NO: 26;
residues 1 to 17 of SEQ ID NO: 27; and
residues 1 to 28 of SEQ ID NO: 28.

23. A homologue of any of the protein fragments of Claim 22.

~~24. An isolated or synthetic DNA encoding a polypeptide fragment according to claim 22.~~

25. A DNA construct which includes a DNA according to claim 24 operatively linked to elements for the expression of said encoded protein.

26. A transgenic plant harboring a DNA construct according to claim 25.

27. The transgenic plant according to claim 26, wherein said plant is a monocotyledonous plant or a dicotyledonous plant.

28. The transgenic plant according to claim 26, wherein said plant is selected from the group consisting of; maize, banana, peanut, field peas, sunflower, tomato, canola, tobacco, wheat, barley, oats, potato, soybeans, cotton, carnations, roses, and sorghum.

~~29. The reproductive material of the transgenic plant according to claim 26.~~

30. A composition comprising an antimicrobial protein according to claim 22 together with an agriculturally-acceptable carrier diluent or excipient.

31. A composition comprising an antimicrobial protein according to claim 22 together with a pharmaceutically-acceptable carrier diluent or excipient.

32. A method of controlling microbial infestation of a plant or mammal, said method comprising treating said plant or mammal with an antimicrobial protein according to claim 22.

33. The method of Claim 32 wherein said mammal is a human.

34. A method of controlling microbial infestation of a plant, the method comprising treating said plant with a composition according to claim 11.

~~35. A method of controlling microbial infestation of a plant, the method comprising introducing a DNA construct according to claim 6 into said plant.~~

36. A method of controlling microbial infestation of a mammal, the method comprising treating the mammal with a composition according to claim 12.

37. The method of claim 19, wherein said mammal is a human.

~~38. A method of controlling microbial infestation of a plant, the method comprising introducing a DNA construct according to claim 25 into said plant.~~

39. A method of controlling microbial infestation of a mammal, the method comprising treating the mammal with a composition according to claim 30.

40. The method of claim 39, wherein said mammal is a human.

41. The method of claim 16, further comprising forming disulphide linkages between said cysteine residues.

IN THE SEQUENCE LISTING:

Please cancel from the application original Sequence Listing pages 34-66 and substitute therefor the attached Replacement Sequence Listing pages 34-58. Please consecutively renumber all pages following the Replacement Sequence Listing.

REMARKS

This Supplemental Preliminary Amendment conforms the Sequence Listing of the priority International Patent Application to the rules of practice specified by the U.S. Patent and Trademark Office. Enclosed herewith are: (1) a paper copy of the Replacement Sequence Listing, and (2) a computer readable version of the Replacement Sequence Listing.

The Specification has been amended to include a reference to the PCT application PCT/AU97/00874, filed December 22, and the priority application. The Specification and Claims have been amended to conform to the rules of practice as specified by the United States PTO and to correct minor informalities. Claims 1-3, 7, 9-16 have been amended. Claims 17-40 have been added. Therefore, Claims 1-40 remain pending. Additionally, the amendment directs